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Figure 2

CONTENTAGEMENT IN CAME IN CASE
GAAGGTAAAC GAAGGTAAAC GAACATCTAGGTATCCAAAGGAGGTC AGATACAGAAGCGTCATCAAAGCATTA AGATACAGAAGCGTCATCAAAGCATTA AGATACAGAAGCGTCATCAAAGCATTA AGATACATTCATCATTCATGT GCAAGCATTTCATGT ATGCACT TAATATCTTTCAGG TAGTTTGATTT GGAGTGATCAAGAAATATGGAAAG ATAGGTTTCATTCAGGAAAGC TAGTTTGATTT TAGTTTCATTCAGGAAACC TAGTTTCATTCATTCAGGAAACC TAGTTTCATTCATTCATTCAGGAAACC TAGTTTCATTCATTCATTCAGGAAACC TAGTTTCATTCATTCATTCATTCATTCATTCATTCATTCA
COGGOGGOGGOATCIGIGAGCIAACAGATICGC COGGOGGOATCIGIGAGCIACAGATICGC COGGOGGOATCIGIGAGCIACAGATICGC COGGOGGOATCIGIGAGCIACAGATICGC COGGOGGOATCIGIGAGCAGCAACAATICGC COGGOGGOGGOATCICIGAATCATACATACATACACAACAACAATICGC COCCOCCAGGOATCICITTICGCATACACATICCTICACATICCTICACATICACATICTICACATI
TITGAAAGT GEITAAAACATCTAGGTATCCAAAAGGAGGTC AT ATATAGATACAGAAGCGTCATCAAAGCATGCC GCAGAAAAACATCGCCGAAGGGCATTA TATTCACT TCTAGGAAGACATCGCCGAAGGGCATTA TATTCACT TCTAGCAAGACATTCATTCATT SAATTGGTAAGGAAGCTTTCATG SAATGGCCAACTCTC CCCTATGCAT CCCTATGCAT CCCACCTTCTCCAAGAACTATGTCTTTCTC GAGTGGATT CCACCTTTCCCAAGAACTATATTGTCTTTCTC CCACCTTCTCCCAAGAACTATATTGTCTTTCTC GAGTGGAATATTCATTTCAT
CICATICICGARGGGAGGGAGGGAGGGAGGGGAGGGCCATITICCGGGGGGGGGG
GACCTICCTITTGGATACCTAGATGTTTTAACAGAAAAGAAA
CGCCGGGGGGATTCCAAGGACGATGCTTACTTATT TAYAGGTAGGCGTATTCCAAGGACATATTTTTTC CGCCGAAGGTTCCAAGTATTCTTTAAAGGATTATTTTTC CGCCGAGGGGGGATATTTCTTAAAGGATTATTTTTC CGCCGAGGGGATATTTCTTAAAGGAATTATTTTCTTTATTGGATATGGATACAGAGGCGTCATCAAGGATGCC GGCTGGCAGGGATATTTTCTTAAAGGAATTTTTTGGATATGAATATAGATACAGAAGGCGTCATCAAGGATTAAGGAAAAAGAATTTCTTTAATGGTTTCCTTTAGTTTCCTTAGTTTCCTTAGTTTCCTTAGTTTCTTAGTTTCTTTAGTTTCTTTAGTTTCTTTAGTTTCTTTAGTTTCTTTAGTTTCTTTAGTTTCTTTAGTTTCTTTAGTTTCTTTAGTTTCTTTAGTTTCTTTAGTTTCTTTAGTTTCTTTAGTTTCTTTAGTTTCTTTAGTTTCTTTAGTTTCTTTTAGTTTCTTTTAGTTTCTTTTAGTTTCTTTTAGTTTCTTTTAGTTTCTTTTAGTTTCTTTTTAGTTTCTTTTTAGTTTCTTTTTAGTTTCTTTTTAGTTTCTTTTTAGTTTCTTTTTTAGTTTCTTTTTAGTTTCTTTTTAGTTTCTTTTTTAGTTTCTTTTTTAGTTTCTTTTTTAGTTTCTTTTTTAGTTTCTTTTTTAGTTTCTTTTTTAGTTTCTTTTTTTAGTTTCTTTTTTAGTTTCTTTTTTAGTTTCTTTTTTAGTTTGTTT
ATTICACT TOTAL AGARANCE CONTRACTOR TECAGAAAAACATCGCCGAAGGCATTA TATTCACT TAT
NAT AATATAGATACAGAGOGTCATCAAAGCATGCC GCCAGAAAAACATCGCCGAAGGGCATTA GTCATAGGAAGCTATGATT TTATTCACT TTATTCACT GAATTGCTAGGAGCTATGATT GAATTGGTAAGAGCCAACTTCATG GAATTGACAACTCTC T GAATTGACAACTCTC GAATTGATAGTTTCTTCTCTC GAATTAGTTTGATTT GAATTGATTGATTTCTTCTCTC ACCTTCTCCAAGAACTATTGGAAAG ACGTTGAGTTTGATTT ACGTTTAGTTTTGATTT ACGTTTAGTTTTTTTTT ACGTTTAGTTTTGATTT ACGTTTAGTTTTGATTTT ACGTTTAGTTTTGATTT ACGTTTAGTTTTGATTT ACGTTTAGTTTTGATTT ACGTTTAGTTTTGATTT ACGTTTAGTTTTGATTT ACGTTTAGTTTTGATTT ACGTTTAGTTTTGATTT ACGTTTAGTTTT ACGTTTAGTTTT ACGTTTAGTTTT ACGTTTAGTTT ACGTTTAGTTT ACGTTTAGTTT ACGTTTAGTTT ACGTTTAGTTT ACGTTTAGTTT ACGTTTAGTTT ACGTTTAGTTT ACGTTT ACGTT ACGTTT ACGTTT ACGTT AC
AI ATATAGATACAGAGCGTCATCAAAGCATGCC ICCAGAAAAAACATCGCCGAAGGGCATTA TATTCACT TATTCACT TATTCACT TATTCACT TATTCACTAGCAACTCTC CAGT GAATTAATATCTTTCAGG AAATTAATATCTTTCAGG AAATTAGTTTGATTTCTCTC CCCTATGCACT TCCACCTTCTCCAAGAACTATTGTCTTTCTCTC CCACCTTCTCCAAGAACTATTGTCTTTCTCTC CCACCTTCTCCAAGAACTATTGTCTTTCTCTC
ATATAGATACAGAAGCGTCATCAAAGCATGCC CCAGAAAAACATCGCCGAAGGGCATTA TATTCACT TCTAGCAAGCATTGCTGTAAATGTCATTCATGT CAGT CA
CCAGAAAAACATCGCCGAAGGCCATTA TICATAGGAAAAACATCGCCGAAGGGCATTA TICACT TOTAGCAAGCTATGATT TCTAGCAAGCATTGCTGTAAATGTCATTCATGT AATTGGTAAGGCCAACTCTC AAATTAATATCTTTCAGG AAATTAATATCTTTCAGG AAATTAGTTTGAGTT CCCTATGCACT CCCTATGCACT AAATTAGTTTGATTT CCCTATGGAATATGGGAAAG TGTTTAGTTTGATTT CCAGGGAATATGGGAAAG ATCAATAGGTACATT
CCAGAAAAAACATCGCCGAAGGGCATTA ATTCACT ATTCACT ATTCACT ATTGGTAAGATTCATGT AATGGTAAGAGGCAAAATTI AATGGTAAGAGCAAAATTI AATGGTAAGAGCAAAATTCATGT AATGGCCAACTCTC AATTGATATCTTTCAGG AATTGATTTCATTCAGG AATTAATATTCTTTCAGG AATTGATTTGAT
CCGGAAAAAACATCGCCGAAGGGCATTA TCATAGGAAGCTATGATT ATTCACT CTAGCAAGCTATGCTGTAAATGTCATTCATG AGT AATGGCCAACTCTC AATGGCCAACTCTC AATGGCAACTCTC CCTATGCAC AGTTAATATCTTTCAGG CCTATGCAC AGTTGATTGCTTTCAGG AGTTGAGTT AGTTGATTCATTCAGG AGTTGAGTT AGTTGATTCATTCAGG AGTTGAGAATATGGAAAG AGTGGAACGT AGTGCAACTT AGTTGATTGATTT AGTTGAATAGGTACATT
TCATAGGAAGCTATGATT TATTCACT TCTAGCAAGCATTGCTGTAAATGTCATTCATGT AATTGGTAAGGGCAGAAGGTCATCCAAAATT AAATTAATATCTTTCAGG AAATTAATATCTTTCAGG SAGTGGAGT SAGTGGAGT SAGTGGATTT SCCTATGCAGAACTATTGTCTTTCTCTC CCACCTTCTCCAAGAACTATTGTCTTTCTCTC CCACCTTCTCCAAGAAATATGGAAAG ATCAATAGGTACATT
STCATAGGAAGCTATGATT TAITCACT TOTAGCAAGCATTGCTGTAAATGTCATTCATGT CAGT AATATGGTAAGAGGCAGAAGGTCATCCAAAATTT AATATGGTAAGAGGCAGAAGGGCAGAATTGTTTTCTCTC GAGTGGAGT TCCACCTTCTCCAAGAACTATTGTCTTTCTCTC TCCACCTTCTCCAAGAACTATTGTCTTTCTCTC TCACCTTTTGGTTTT TCATTAGGTACATT TCATTAGGTACATT TCATTAGGTACATT TCATTAGGTACATT TCATTAGGTACATT TAITCATTAGGTACATT TAITCATTAGGTACATT
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ACGGACGCGGAGGATGCTANAGAATTCTTGCT CAACGACGCGGGAGGTGCTAAAGAAATTCTTGCT CAACGACGCGGGAGGTGCTAAAGAAATTCTTGCT CAACGACGCGGGAGTTGCTGAACGTTAACTAATTATTGGTCTAAGCAGTTGCTGTAAATTCTTTGCT CAACGACGCGAGGATTGCTAAACATCATCTAATTTGCTTAATTGGTCTAAGGCAGTGCTAATTGCTTAACGTTACCGTTAATTTTACATTATTTACATTATTTACATTATTTACATTATT
SETCTAGGAAGCATTTGCTGTAAATGTCATTCATGT CCAGT AAATATGGTAAGAGGCAGAAGGTCATCCAAAATTC AGAATGGCCAACTCTC GT TGAAATTAATATCTTTCAGG TTCCCTATGCACT TTCCCTATGCACT TTCCACCTTTCCAGAACTATTGTCTTTCTCTC TTCCACCTTTCAGAACTATATTGTCTTTCTCTC TTCCACCTTTGATTT AACAGTACATTGATTT AACAGTACATTGATTT AAATCAATAGGTACATT
GCAATITIGGATGACCTCTGCCTCTACCATATTGACTTCACGT GGCAATITIGGATGACTTCTGCCTCTACCATATTGACTTCACGT GGCGCGGGATATGTAAAATAAGTACCGTTAA GGCGCGGGGATACTGATGATTGTGTGATGATGATGGTAGATGGTAGGTA
CGCGCGAGGATATGTAAAATAAGTACGTTAA AGACATACGAAGGATACTGAATATGGTGAAATATGGTAAGGAGGAGAGGCAGAAGGTCAAAATATGCAAAATATGCAAAATATGCTAATATG AGACATAGGCATTCATTACAATATTGGTGAATATTGGTGAATATGGTAATATGCAACAGAAGTTGGTTG
AAATCAATAGGTAATTCTCGG TTGAAATTAATATCTTTCAGG TTCACCTATGCACT ATTCCACCTTCTCCAAGAACTATTGTCTTTCTCTC TTCACACTTCTCCAAGAACTATTGTCTTTCTCTC TATTCCACCTTCTCCAAGAACTATTGGAAAG TAAATCAATAGGAAAG TAAATCAATAGGAAAG
CGCGCCGAGGGTAGGTTTACTTCTGTTGG CGTGCCAACAGGTAGAGTCAACCAAACCA
CATGCCAACAGAAGGTCAACTACAAGTCAACCAACCATACAAGGCCAACTCTC CCTGAAGATTAATTCAAGATGAAAGAGGACAGTTGTTGT CCTGAAGATTTAATTCAAGATGAAAGAGGACAGTTGTTGT CCAGTGGATCCAGCAACCAACAACTGTCCCTTTCTATCTTGAAATTAATT
GGT CTTGAAATTAATATCTTTCAGG CTTCCCTATGCACT ACTGAGTGAGT ATTCCACCTTCTCCAAGAACTATATTGTCTTTCTCTC CATAGTTTAGTT
CITGAAATTAATATCTTTCAGG CITCCCTATGCACT ACTGAGTGGAGT ATTCCACCTTCTCCAAGAACTATATGTCTTTCTCTG ATTCCACGTTTGATTTGA
CITICACTATGCACT ACTGAGTGGAGT ATTCCACCTTCTCCAAGAACTATATTGTCTTTCTCTG SCTATGTTTAGTTTGATTT GAACAGTGGAGTGATCAAGAAATATGGAAAG TTAAATCAATAGGTACATT
CITCCCTATGCACT ACTGAGTGGAGT ATTCCACCTTCTCCAAGAACTATATTGTCTTTCTCTG ACTATGTTTAGTTTGATTT GAACAGTGGAGTGATCAAGAAATATGGAAAG TAAATCAATAGGACATT
ITCCCTATGCACT CTGAGTGGAGT ITCCACCITCTCCAAGAACTAIATTGTCTTTCTGG CTATGITTAGTTTGATTT SAACAGTGGAGTGATCAAGAAATATGGAAAG TAAATCAATAGGTACATT
CTGAGTGGAGT TTCCACCTTCTCCAAGAACTATATTGTCTTCTCTG TTCCACCTTTCTCCAAGAACTATATTGTTTTCTCTG TATGTTTAGTTTGATTT AACAGTGGAGTGATCAAAATATGGAAAG AAATCAATAGGTACATT
TCCACCTTCTCCAAGAACTATATTGTCTTTCTCTG IATGITTAGTTTGA1TT ACAGTGGAGTGATCAAGAAATATGGAAAG WATCAATAGGTACATT
TCCACCITCTCCAAGAACTATATTGTCTTTCTCTG ATGITTAGTTTGATTT ACAGTGGAGAACAAATATGGAAAG AATCAATAGGTACATT
ATGTTTAGTTTGATTT ACAGTGGAGTGATCAAGAAATATGGAAAG AATCAATAGGTACATT
ATGTTTAGTTTGATTT ACAGTGGAGTGATCAAGAAATATGGAAAG AATCAATAGGAAAG
ATGITTAGTITGATIT ACAGTGGAGTGATCAAGAAATATGGAAAG ATCAATAGGTACATT
ACAGTGGAGTGATCAAGAAATATGGAAAG WATCAATAGGTACATT
AACAGTGGAGTGATCAAGAAATATGGAAAG AAATCAATAGGTACATT
AACAG I GGAGI GATCAAGAATA I GGAAAG AAATCAATAGGTACATT
IAAAICAAIAGGIACAII
COCCOCA A A TITICA TO A TOTAL CAN THAT TO THE CONTRACT TO TAXATA TA COLOR A COLOR A A COLOR A A TITICA TAXATA TA COLOR A COLOR
<u>GOCCITICADA ITTORAGANI INTONICANI NA SECONO CONTROLO INTONICANA PORTACO CANA PORTACO CONTROLO CONTROL</u>

Figure 2 cont'd

probe/DM	probe/DM		9292	CGCGCCGAGGAGACATCTCCAAGTTTGC	294500
synthetic target	synthetic target	ĕ	CTCTGCAAACTT(<u>CICTGCAAACTTGGAGATGTCTTATTACCAAAAATAGAAAATTAGAGAGTCACTTTTAGTATGCTCAATCTGAATTTGAAAGGCACATC</u>	1010000
	invader		GCTCACCTGTGGT	GCTCACCTGTGGTATCACTCCAAAGGCTTTCCTA	345000
3 hex probe/DM cececceAGGTC/	probe/DM		CGCGCCGAGGTCA	CGCGCCGAGGTCACTGTTGCAAAGTTATG	327800
3 none synthetic target GATTCAATAACTT	synthetic target	let	GATTCAATAACTT	GATTCAATAACTTTGCAACAGTGAAGGAAAGCCTTTGGAGTGATACCACAGGTGAGCAA	683000
2 none invader CAAGAGTCTTCC/	invader		CAAGAGTCTTCC	CAAGAGICITCCATCTGTTGCAGTATTAAAATGGA	390000
2 hex probe/DM CGCGCCGAGG	probe/DM		cececceaee	CGCGCCGAGGTGAGACACCCTGAAA	327400
let	synthetic target	let	TTCCTTTCAGG	TTCCTTTCAGGGTGTCTTACTCACCAT1TTAATACTGCAACAGATGGAAGACTCTTG	601000
4 none invader CATTTACAGCA	invader		CATTTACAGCA	CATTIACAGCAAATGCTTGCTAGACCAATAATTAGTTATTCACCTTGCTAAAGAAATTCTTGCTG	
Pex hex probe/DM CGCGCCGAGGC	probe/DM		CGCGCCGAGGC	CGCGCCGAGGCATTGACCTCCACTCAGT	
4 none synthetic target ACTGAGTGGAG	synthetic target	jet	ACTGAGTGGAG	ACTGAGTGGAGGTCAATGAGCAAGAATTTCTTTAGCAAGGTGAATAACTAATTATTGGTCTAGCAAGCA	
A none invader TCCAAGTTTGCA	invader		TCCAAGTTTGC/	TCCAAGTITGCAGAAAGACAATATAGTICTITC	
4 hex probe/DM CGCGCCGAGG	probe/DM		CGCGCCGAGG	CGCGCCGAGGGGAGAAGGTGGAATCACA	
4 none synthetic target TGTGATTCCAC	synthetic target	jet	TGTGATTCCAC	TGTGATTCCACCTTCTCAAAGAACTATATTGTCTTTCTCTGCAAACTTGGA	
3 none invader CCTTCATCACAT	invader		CCTTCATCACAT	CCTTCATCACATTGGAATGCAGATGAGAATAGCTATGTTTAGTTTGATTTATAAGAAGC	664000
3 hex probe/DM CGCGCCGAGG	probe/DM		CGCGCCGAGG	CGCGCCGAGGTTAATACTTCCTTGCACAGG	311900
3 none synthetic target GGGGCCTGTG	synthetic target	jet	GGGCCTGTG	GGGGCCTGTGCAAGGAAGTATTAACTTCTTATAAATCAAACTAAAACATAGCTATTCTCATCTGCATTCCAATGTGATGAAGGCCAA	965000
2 none invader CGCAGAACAA	invader		CGCAGAACAA	CGCAGAACAATGCAGAATGAGATGGTGAATATTTTCCT	467000
2 hex probe/DM cccccsAcc	probe/DM		CGCGCCGAGG	CGCGCCGAGGAGGATGCTTTGATTA	336800
2 none synthetic target TGCACTAATCA	synthetic target	get	TGCACTAATCA	TGCACTAATCAAAGGAATCATCCTCTGGAAAATATTCACCACCATCTCATTCTGCATTGTTCTGCG	703000
Exemplary 3' blocking	y 3' blocking				82,500
Pool group Oligo type (Arm)	Oligo type (Ar	Oligo type (Arm)		Sequence (5'-3')	M.¹ cm.¹
all none Invader tgtacttcatgctgtcta	Invader		tgtacttcatgctgtcta	tgtacticatgctgtcdacactaagagaatgagagacacaca	503500
all hex Probe/SNP4b tccgcgcgtcctgaa	Probe/SNP4b	q	teegegegteetgaa	tecgegetectgaagaageaceaateatg	321200
all none Synthetic Target (ttcatgattggtgcttct	Synthetic Target	get	tttcatgattggtgcttct	tticatgattggtgettetteagtgtgteteteattetetettagtgtagaeageageagaagtacattt	698200

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-ggc-gcg-hex	-gcg-tcc-gt-hex	2-9c9-c99-a-hex	
Y-tct-X-agc-cgg-ttt-tcc-ggc-tga-gac-ctc-c	Y-tct-X-agc-cgg-ttt-tcc-ggc-tga-gac-tcc-c	Y-tct-X-tcg-gcc-ttt-tgg-ccg-aga-gag-gac-	X = Onencher = 728
DM/FAM	ER24/FAM	SNP4b/Red	
Hex	Hex	Hex	
all	1, 2, 5	all	

X = Quencher = 228 Y = Dye = FAM for 1055-48-08 and 1055-48-09 and Y = 235 (or Redmond Red) for 1055-49-04

		3' blocking			823
Mutation	Pool	group	Oligo type (Arm)	Sequence (5-3')	M-1 cm-1
delF508	delF508	none	Invader	TGATGACGCTTCTGTATCTATATTCATCGTAGGAAACACA	441500
delF508	delF508	Hex	WT Probe	CGCGCCGAGGCAAAGATGATATTTCTTTAATGGT	382200
delF508	delF508	Нех	Mut Probe	AGCTCGTCCGACCACAATAATATTTCTTTAATGGTGCCA	418100
delF508	delF508	Hex	DM/FAM	Y-tct-X-agc-cgg-tft-tcc-ggc-tga-gac-ctc-ggc-gcg-hex	347150
delF508	delF508	Hex	Wingra/Red	Y-tct-X-tcg-gcc-ttt-tgg-ccg-aga-gat-gtc-gga-cga-gct-hex	390400
delF508	delF508	none	WT Target	TGCCTGGCACCATTAAAGAAAATATCATCTTTGGTGTTTCCTATGATGAATATAGATACAGAAGCGTCATCAAA	837500
delF508	delF508	попе	Mut Target	ATGCCTGGCACCATTAAAGAAAATATCATTGGTGTTTCCTATGATGAATATAGATACAGAAGCGTCATCAAA	828100

		3' blocking		
Mutation	Pool	group	Oligo type (Arm)	Sequence (5'-3')
2184delA	2184delA	none	Invader	CTTCCTTTTTTCCCCAAACTCTCCCAGTCTGTTTAAAAGATTGTTTA
2184delA	2184delA	hex	MUT probe/DM	CGCGCCGAGGTTTGTTTCTGTCCAGGAG
2184delA	2184delA	hex	WT probe/ER24	ACGGACGCGGAGTTTTGTTTCTCCAGGAG

X = Quencher = 228 $\,$ Y = Dye = FAM for 1055-48-08 and Y = 235 (or Redmond Red) for 1144-16-02

ACGGACGCGGAGAATTCATCATTGTTCAGG	ACGGACGCGGAGTGAGTAAGACCCCTGAAA	
probe/ER24	probe/ER24	
hex	hex	
2	2	
711+1G>T	3849+10kb	

Fig. 3

	Mutation	Sample	IC ALLELE	MUT ALLELE	FOZ Ratio
A	2789+5G>A	26mix	3.94	4.69	1.19
\mathbf{A}	R1162X	29	3.42	2.18	0.62
	R347P	15	3.38	4.60	1.36
	G85E	21	3.62	2.55	0.70
	R560T	9	3.30	2.47	0.75
	dell507	1	3.16	1.98	0.63
	1898+1G>A	111 A2/8	6.23	2.84	0.46
	R117H	30	3.46	1.87	0.54
	delF508 homo MT	3	3.44	1.14	0.33
	WT gDNA	03-243	3.58	1.06	0.30
	Wight	03-243	3.30		0.50
	Mutation	Sample	IC ALLELE	MUT ALLELE	FOZ Ratio
	2184delA plasmid/internal	oup.o			. 02
	control syn. Target	plasmid/syn. Target	4.67	3.65	0.78
	Mutation	Sample	IC ALLELE	MUT ALLELE	FOZ Ratio
B	A455E	8	3.26	2.88	0.88
D	3659delC	· 14	3.38	2.36	0.68
	N1303K	16	3.92	2.11	0.54
	3120+1G>A	6	3.84	2.45	0.64
	G551D	20	3.44	2.04	0.59
	WT gDNA	03-243	3.74	1.00	0.27
	I148T/Internal control	syn. target	4.35	5.08	1.17
	1078delT/Internal control	syn. target	4.44	4.97	1.12
				·	
	Mutation	Sample	IC ALLELE	MUT ALLELE	FOZ Ratio
\boldsymbol{C}	711+1G>T	2	3.95	2.82	0.71
	W1282X	19	4.44	2.16	0.49
	1717-1G>A	. 28	4.87	2.19	0.45
	3849+10kbC>T	5	3.82	2.48	0.65
	WT gDNA	03-243	4.67	1.10 ·	0.24
			10 41 : 5: 5	AUTALIE	E07 5 ::
_	Mutation	Sample	IC ALLELE	MUT ALLELE	FOZ Ratio
D	621+1G>T	11	4.23	2.05	0.49
	G542X	18	3.40	2.83	0.81
	R553X	7	4.53	3.27	0.72
	R334W	22	3.72	2.79	0.75
	WT gDNA	03-243	4.18	1.14	0.27